An **Examiner's Amendment** to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this Examiner's Amendment was given in a telephone interview with Mr. Daniel J. Pereira on 5/8/2008.

Examiner's Amendment to the Claims

In claim 23, after "claim", delete "10" and substitute therefor --- 22 ---.

Examiner's Amendments to the Specification

In page 11, line 21, please insert the following: ----

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1:

A. Expression experiments in Example 1 are shown where after induction with IPTG, the recombinant protein is strongly expressed in the bacteria transformed with the plasmid pET-15b containing the Arabidopsis cDNA insert.

pET-15b = bacteria transformed with the nonrecombinant plasmid (negative control) pET-15b+insert=bacteria transformed with the recombinant plasmid,

- = no induction with IPTG,

+ = induction with IPTG,

*= band corresponding to the recombinant 41 kDa protein.

B. Solubilization of the Recombinant Protein in Example 1 is shown where the recombinant protein (*) is found in 2 different fractions: a water-soluble fraction present in the E. coli cytosol and an insoluble fraction, which is not solubilized by Triton X-100, which indicates that it is probably aggregated in the form of inclusion bodies:

pET-15b=bacteria transformed with the nonrecombinant plasmid (negative control),

pET-15b+insert=bacteria transformed with the recombinant plasmid,

S=soluble proteins,

D=proteins solubilized in Triton X-100,

I=proteins not solubilized in Triton X-100,

*=band corresponding to the recombinant 41 kDa protein.

FIG. 2: Purification of the Recombinant Protein in Example 1 is shown:

C=bacterial pellet diluted in the lysis buffer (10 µl),

S=soluble bacterial proteins (10 µl),

P=proteins not bound (10 µl),

L, L1, L2=washes with the lysis buffer (35 and 60 mM imidazole) (15 µl),

Elution=fraction eluted in the presence of 250 mM imidazole.

FIG 3: Analysis of the Chloroplast Fractions in Example 1 is shown:

A. The 41 kDa protein is associated only with the chloroplast envelope and is not detected in the stroma or in the thylakoids.

C=chloroplast proteins,

E=envelope membrane proteins,

S=stroma proteins,

T=thylakoid membrane proteins.

B, C The 41 kDa protein is not affected by the treatment with thermolysin (FIG. 3B), whereas the same proteolytic treatment carried out on solubilized envelope proteins shows the sensitivity of the 41 kDa protein to the thermolysin treatment (FIG. 3C).

0=absence of thermolysin,

20=thermolysin at 20 µg/ml,

50=thermolysin at 50 µg/ml,

100=thermolysin at 100 µg/ml.

D. The 41 kDa protein is found, like the IE18 protein, only in the preparations of chloroplast envelopes enriched in inner membrane.

E=envelope membrane proteins,

OM=outer membrane proteins,

IM=inner membrane proteins.

FIG. 4 Analysis of the Interactions Between the IE41 Protein and the Inner Membrane of the Chloroplast Envelope

A. The analyses by SDS-PAGE and Western blotting of the treated envelope fractions show that the IE41 protein is not solubilized after sonication of the envelope vesicules. On the contrary, the major soluble proteins of the stroma (RbcL) which are sequestered

in the envelope vesicles, and which are known to contaminate the envelope fractions, are solubilized by this treatment. This shows that the IE41 protein is neither a soluble protein of the intermembrane space, nor a soluble protein of the stroma that contaminates the purified envelope fraction.

- = no sonication,

+=sonication,

S=soluble proteins, I=membrane proteins.

B. These results show that the IE41 protein is at least partly solubilized by the salt (KI, NaCl) or moderately alkaline (Na.sub.2CO.sub.3) treatments, which have no effect on the intrinsic protein IE18, it only being possible for the latter to be solubilized by a strong alkaline treatment (NaOH).

+=sonication (10 sec),

NaCl 0.5 M=treatment 1,

KI 0.5 M=treatment 2,

0.1 M Na.sub.2CO_3,

pH 11=treatment 3,

0.1 N NaOH=treatment 4,

S=soluble protein fraction,

I=insoluble protein fraction.

C. These results show that the IE41 protein can be completely solubilized with concentrations of Triton X-100 (0.2%) that are much lower than those (of the order of 2%) which are necessary for solubilizing the intrinsic proteins.

Mix=purified envelope vesicles,

M=envelope membrane proteins,

S=envelope soluble proteins.

FIG. 5: Immunopurification of the Spinach IE41 Protein. The E2 fraction comprising the purified natural spinach IE41 protein (So) remains contaminated by the recombinant Arabidopsis (His-tag)-IE41 protein.

A: analysis by SDS-PAGE;

B: Western blotting;

Mix=solubilized envelope proteins;

C=insoluble proteins;

S=soluble proteins;

L1, L2, L3=fractions recovered in the course of the 3 successive washes;

E1=fraction eliminated by incubation with the Ni-NTA resin;

E2=purified spinach IE41 protein (So)+(His-tag)-IE41.

FIG. 6: the cDNA Encoding the Spinach IE41 Protein.

FIG. 7: Arabidopsis and spinach IE41 proteins aligned with homologous proteins from a bacterium, from a yeast and from animals. Arabidopsis thaliana: IE41 ATH (SEQ ID NO: 1), Spinach: IE41 SOL (SEQ ID NO: 3); Esherichia coli: QORECOLI, (SEQ ID NO: 6) Saccharomyces cerevisiae: QORYEAST, (SEQ ID NO: 7) Cavia Porcellus:

QORCAVPO, (SEQ ID NO: 8) Mouse: QORMOUSE. (SEQ ID NO: 9). The residues conserved in the 6 peptide sequences are highlighted in dark gray. The residues conserved in the IE41 sequence and in at least one other homologous protein sequence are highlighted in light gray. The similarities between residues are based on the following groups: ASPTG, ILMV, KRH, NQ, DE, YWF and C.

FIG. 8: Constructs used in Example 5, IE41=plasmid 35Ω-IE41-sGFP(S65T); Δ(1-31)IE41=plasmid 35 Ω.- Δ (1-31)IE41-sGFP(S65T); Δ (1-59)IE41=plasmid 35 Ω -Δ (1-59)IE41-sGFP(S65T): Δ (1-99)IE41=plasmid 35 Ω -Δ (1-99)IE41-sGFP(S65T); (6-100)IE41=plasmid 35 Ω -(6-100)IE41-sGFP(S65T); (60-100)IE41=plasmid 35 Ω -(60-100)IE41-sGFP(S65T)

FIG. 9: In Planta Analysis of the Plastid-Targeting of the IE41 Protein. A: SDS-PAGE analysis; B: Western blotting with the anti-GFP antibody: the black arrows indicate the presence of the GFP protein in the fusions expressed in Arabidopsis; C: Western blotting with an anti-IE41 antibody: the black arrows indicate the presence of the IE41 protein in the fusions expressed in Arabidopsis; the white diamond indicates the position of the natural IE41 protein present in all the extracts; WT=non-transformed plant; M=molecular weight markers; GFP=plasmid 35 Ω -sGFP(S65T); TP GFP=plasmid 35 Ω -TP-sGFP(S65T); IE41 GFP=plasmid 35 Ω -IE41-sGFP(S65T); Δ (1-59)IE41 GFP=plasmid 35 Ω.- Δ.(1-59)IE41-sGFP(S65T); Δ (1-99)IE41 GFP=plasmid 35 Ω -Δ (1-

99)IE41-sGFP(S65T); (6-100)IE41 GFP=plasmid 35 Ω -(6-100)IE41-sGFP(S65T); (60-100)IE41 GFP=plasmid 35 Ω -(60-100)IE41-sGFP(S65T).

FIG. 10: The subcellular location of the proteins expressed by the various constructs was visualized by fluorescence microscopy, as described in example 5. GFP=plasmid 35 Ω.-sGFP(S65T); TP-RBCS GFP=plasmid 35 Ω -TP-sGFP(S65T); IE41 GFP=plasmid 35.OMEGA.-IE41-sGFP(S65T); (6-100)IE41 GFP=plasmid 35 Ω -(6-100)IE41-sGFP(S65T). ----.

The following is an **Examiner's Statement of Reasons for Allowance**:

Claims 13-35 remain allowed for the reasons of record.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maryam Monshipouri whose telephone number is (571) 272-0932. The examiner can normally be reached on Tues.-Fri., from 7:00 a.m to 5:30 p.m..

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleene Kerr Bragdon can be reached on (571) 272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Maryam Monshipouri/

Primary Examiner, Art Unit 1656

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